

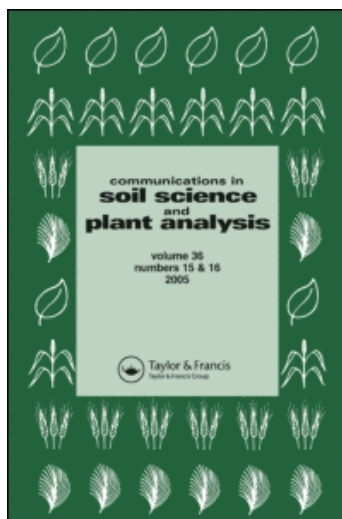
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## Communications in Soil Science and Plant Analysis

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title-content=t713597241>

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Online Publication Date: 01 May 2009

**To cite this Article** Dell, Curtis J.(2009)'Potential for Quantification of Biologically Active Soil Carbon with Potassium Permanganate',Communications in Soil Science and Plant Analysis,40:9,1604 — 1609

**To link to this Article:** DOI: 10.1080/00103620902831685

**URL:** <http://dx.doi.org/10.1080/00103620902831685>

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## Potential for Quantification of Biologically Active Soil Carbon with Potassium Permanganate

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**Abstract:** Oxidation of soils with 333 mM potassium permanganate ( $\text{KMnO}_4$ ) has been identified as a means to quantify labile soil carbon (C) and may have potential for rapid measurement of biologically active soil C. In the current study, active C pools in several soils were estimated by oxidation with a range of  $\text{KMnO}_4$  concentrations and compared to estimates determined from carbon dioxide evolution during 28-d incubations. Digestion with 333 mM  $\text{KMnO}_4$  identified larger active C pools than did incubation data. However, shaking soils for 15 min with 2.5 mM  $\text{KMnO}_4$  provided estimates of active C that accounted for 1.5 to 1.9% of the total C pools and were not significantly different than those estimated using incubation data. Oxidation with dilute  $\text{KMnO}_4$  appears to be feasible to rapidly quantify active soil C pools. However, measurements are dependent on  $\text{KMnO}_4$  concentration and shaking time, so great care is needed to assure consistent results.

**Keywords:** Active soil C, carbon dioxide, potassium permanganate

Describing the cycling of C through soils often requires the partitioning of soil organic C (SOC) among pools with varying turnover times. A three-pool model is frequently used where the organic C compounds persist in an “active” pool for days to a few months, in a “slow” pool for up to several decades, and in a “resistant” pool for centuries (Parton, Stewart, and Cole

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Received 29 May 2007, Accepted 29 November 2007

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1988). Active C is generally quantified by measuring carbon dioxide ( $\text{CO}_2$ ) evolution during laboratory incubations under optimal conditions (Paul, Morris, and Böhm 2001). However, incubations require several weeks to complete. Additionally,  $\text{CO}_2$  evolution rates are dependent on the determination of the proper soil moisture and length of incubation, and  $\text{CO}_2$  leaks during incubation can cause measurement errors.

The measurement of C released by the oxidation of soil with 333 mM potassium permanganate ( $\text{KMnO}_4$ ) has been proposed by Blair, Lefroy, and Lisle (1995) as a rapid means to quantify labile organic C in soils. The labile C pools identified by Blair, Lefroy, and Lisle (1995) comprised 20–30% of the total SOC, which is a much greater proportion of the total C pool than expected for the active C pool described. Wang et al. (2003) found a statistically significant correlation between  $\text{CO}_2$ -C respired during 7-d incubations (23 soils) and the quantity of C oxidized by 333 mM  $\text{KMnO}_4$ , but the pool of C identified by  $\text{KMnO}_4$  was 2.4 to 10.4 times larger than that identified by incubation. This suggests that 333 mM  $\text{KMnO}_4$  oxidized a significant portion of the slow C pool, in addition to active C. Weil et al. (2003) presented a modified method, using 20 mM  $\text{KMnO}_4$ , which quantified an active SOC pool that was more highly correlated with substrate-induced respiration, basal respiration, microbial biomass, and soluble carbohydrates than pools identified with 333 mM  $\text{KMnO}_4$ .

The current study was conducted to compare active SOC pools identified by  $\text{CO}_2$  evolution during soil incubation with those quantified by oxidation with 333 mM and more dilute  $\text{KMnO}_4$  to assess the feasibility of using oxidation with  $\text{KMnO}_4$  as an alternative to incubations. Although a chemical assay would not directly measure the rate of C turnover, a rapid chemical method would be a valuable tool for studies where the size of the active C pool is needed.

Soils were obtained from the upper 5 cm of four experimental sites with contrasting management and total SOC (TOC) contents (Table 1). Soil 1a was a Hagerstown silt loam (fine, mixed semiactive, mesic Typic Hapludalf) where corn had been grown continuously without manure applications, and soil 1b was from the same site but with annual manure applications since 1982. Soil 2a was a Hagerstown silt loam where first-year corn was grown following hay with no manure additions, and soil 2b was obtained from the same site but where manure was applied 6 weeks prior to sampling. Soil 3 was a Hublersburg silt loam (clayey, illitic, mesic Typic Hapludult) that had been maintained as a grazed pasture for more than 20 years. Soil 4 was an Othello silt loam (fine-silty, mixed, active, mesic Typic Endoaquults) where a rotation of corn and soybeans was grown. Field-moist soil was passed through a 6-mm screen to remove large stones or root fragments. A subsample of each soil was air dried, crushed, and sieved through a 2-mm sieve, and TOC was determined by combustion in an EA 1100 elemental analyzer (CE Elantech, Lakewood, N.J.).

**Table 1.** Quantification of active soil organic C pools identified by either CO<sub>2</sub> evolution during 28-d incubations or oxidation with 2.5 mM or 333 mM KMnO<sub>4</sub>

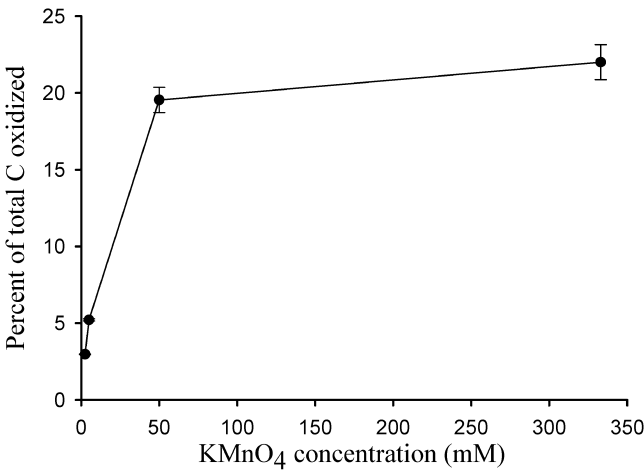
Soil	KMnO <sub>4</sub> -oxidized C						
	Respired C		2.5 mM		333 mM		
	TOC (g kg <sup>-1</sup> )	mg kg <sup>-1</sup> Percentage of total TOC	mg kg <sup>-1</sup> Percentage of total TOC	mg kg <sup>-1</sup> Percentage of total TOC	mg kg <sup>-1</sup> Percentage of total TOC	mg kg <sup>-1</sup> Percentage of total TOC	mg kg <sup>-1</sup> Percentage of total TOC
Soil 1a	12.7	275 ± 22 a 2.2	204 ± 8 a 1.6	204 ± 8 a 1.6	3.6 ± 0.5 b 28.7	3.6 ± 0.5 b 28.7	3.6 ± 0.5 b 28.7
Soil 1b	17.2	365 ± 27 a 2.1	279 ± 21a 1.7	279 ± 21a 1.7	4.2 ± 0.7 b 24.9	4.2 ± 0.7 b 24.9	4.2 ± 0.7 b 24.9
Soil 2a	17.7	285 ± 49 a 1.6	268 ± 13 a 1.5	268 ± 13 a 1.5	3.3 ± 0.4 b 18.7	3.3 ± 0.4 b 18.7	3.3 ± 0.4 b 18.7
Soil 2b	17.3	392 ± 23 a 2.3	254 ± 4 a 1.5	254 ± 4 a 1.5	3.8 ± 0.4 b 21.8	3.8 ± 0.4 b 21.8	3.8 ± 0.4 b 21.8
Soil 3	34.0	951 ± 166 a 2.8	625 ± 117 a 1.9	625 ± 117 a 1.9	6.3 ± 1.0 b 18.7	6.3 ± 1.0 b 18.7	6.3 ± 1.0 b 18.7
Soil 4	12.6	385 ± 8 a 3.1	219 ± 10 a 1.7	219 ± 10 a 1.7	4.4 ± 0.2 b 34.7	4.4 ± 0.2 b 34.7	4.4 ± 0.2 b 34.7

*Notes.* Values are expressed as both the mean C concentration plus or minus the standard error (n = 4) or as the percentage of the total organic C (TOC) represented by the mean concentration. Mean C concentrations for the same soil that are accompanied by the same letter do not differ among measurement methods (P ≤ 0.05).

For incubations, sufficient water was added to 50-g moist soil subsamples to maintain water content at 55% water-filled pore space ( $0.25\text{--}0.28\text{ g g}^{-1}$ ). Soils were incubated at  $23\text{ }^{\circ}\text{C}$  for 28 d. [Although much longer incubation times are generally used, the active pool size estimated by fitting long-term incubation data to a three-pool model is typically very similar to cumulative  $\text{CO}_2$  evolution during the first 28 d of incubation (Franzluebbers et al. 2000; Dell, unpublished data)]. Soils were incubated within 1-L Mason jars sealed with lids that were fit with a rubber septum to allow headspace gas sampling by needle and syringe. Carbon dioxide concentrations in jar headspaces were measured every 7 d with an infrared gas analyzer (LiCorr, Lincoln, Neb.), and  $\text{CO}_2$  concentrations within jars were corrected for background  $\text{CO}_2$  concentration. Active SOC pools, indicated by cumulative  $\text{CO}_2$  evolution during 28-d incubations, accounted for 1.6 to 3.1% of TOC for the soils tested (Table 1).

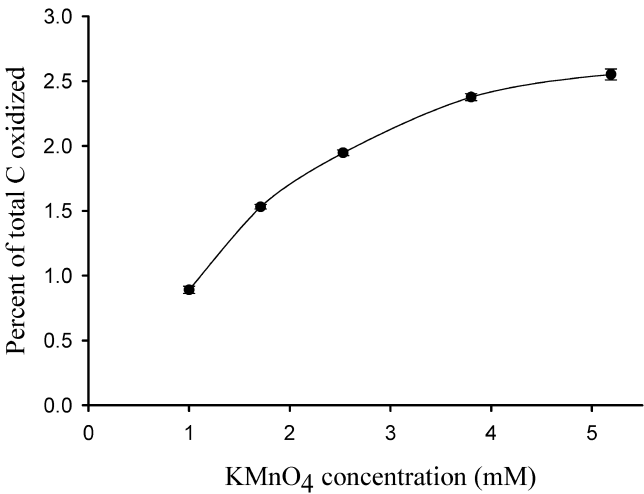
To determine a concentration of  $\text{KMnO}_4$  that identified a pool of C that most closely matched the size of the active C pool identified by incubation, quantities of C oxidized by a range of  $\text{KMnO}_4$  concentrations were measured in soil 2a ( $n = 4$ ). Bottles containing 50 mL of  $\text{KMnO}_4$  solution (either 333, 50, 5, or 2.5 mM) and the quantity of soil that contained 30 mg of SOC were shaken at 250 rpm on an orbital shaker for 1 h. After shaking, samples were centrifuged at 3500 rpm for 5 min. An aliquot of solution was then diluted 1:2000 (333 mM  $\text{KMnO}_4$ ), 1:200 (50 mM  $\text{KMnO}_4$ ), or 1:20 ( $\leq 5$  mM  $\text{KMnO}_4$ ) with deionized water, and absorbance was determined at 565 nm. Calibration standards were treated similarly. The quantity of C oxidized was calculated assuming 1 mmol of permanganate ( $\text{MnO}_4$ ) was consumed in the oxidation of 0.75 mmol of C. Because C oxidized by all  $\text{KMnO}_4$  concentrations during a 1-h digestion exceeded the quantity of active C measured during incubations (1.6% of TOC) (Figure 1), additional digestions were carried out with 1.0, 1.75, 2.5, 3.75, and 5.0 mM  $\text{KMnO}_4$  with shaking time reduced to 15 min. Oxidation with 2.5 mM  $\text{KMnO}_4$  for 15 min provided an estimate of active C that closely matched the pool size indicated by incubation (Figure 2). The other five soils were then oxidized with 333 (1-h shaking time) and 2.5 mM  $\text{KMnO}_4$  (15-min shaking time) using the soil-to-solution ratio indicated previously ( $n = 4$  per soil).

Pools of active C estimated using 28-d incubations and 2.5 mM  $\text{KMnO}_4$  were not significantly different ( $P \leq 0.05$ ) for any of the soils tested (Table 1), but the mean quantities of C oxidized by 2.5 mM  $\text{KMnO}_4$  were always somewhat less than those determined from incubation. Also, the correlation between C pool sizes estimated using the two methods was not as close for the other soils as was the original comparison for soil 2a. This observation indicates that a slight modification of  $\text{KMnO}_4$  concentration might be needed to optimize the quantification of active C for individual soils. Although similar quantities



**Figure 1.** Active soil organic C pools in soil 2a identified by digestion for 1 h with four  $\text{KMnO}_4$  concentrations. Use of all four  $\text{KMnO}_4$  concentrations identified active C pools that were larger than those identified using 28-d incubations (1.6% of total organic C). Error bars are the standard error ( $n = 4$ ).

of active C that were identified with dilute  $\text{KMnO}_4$  and 28-d incubation, it is not known if the C pools identified by the two methods are structurally identical. Confirmation of the structural similarity of these C pools would be difficult, but it is very likely that similar organic compounds identified by assuming susceptibility to decomposition in



**Figure 2.** Active soil organic C pools in soil 2a identified by digestion for 15 min with five very dilute  $\text{KMnO}_4$  concentrations. Error bars are the standard errors ( $n = 4$ ).

dilute  $\text{KMnO}_4$  and by microbial enzymes are indeed similar. As expected, oxidation of soil with 20 and 333 mM  $\text{KMnO}_4$  quantified substantially larger C pools (up to 28.7% of TOC) than those identified by incubation.

Although oxidation of soils with 333 mM  $\text{KMnO}_4$  (Blair, Lefroy, and Lisle 1995) or 20 mM  $\text{KMnO}_4$  (Weil et al. 2003) clearly are not appropriate to determine the biologically active C pool described in the three-pool C partitioning model (Parton, Stewart, and Cole 1988), the use of very dilute  $\text{KMnO}_4$  has potential as a rapid substitute for incubation. Over the range of TOC tested (12.6 to 34.0 g C kg<sup>-1</sup>), estimates of active C did not differ significantly when determined by digestion for 15 min with 2.5 mM  $\text{KMnO}_4$  or from  $\text{CO}_2$  evolution during a 28-d incubation. The digestion method is rapid and reproducible and can be easily adapted to analyze large numbers of samples. However, researchers who consider switching from incubation to digestion methods should first consider the limitations and potential complications. Measurements are very dependent on  $\text{KMnO}_4$  concentration and shaking time, so great care is needed to assure accurate reagent preparation and consistent laboratory procedures. Because the correlation of factors such as clay type and content with quantities of  $\text{KMnO}_4$ -digestible C is not known, calibration to determine the best  $\text{KMnO}_4$  concentration for a specific soil is advisable.

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